Reversed-Phase HPLC Columns

Hamilton reversed-phase HPLC columns combine the best characteristics of silica-based and polymeric columns to arrive at a product that is highly inert and long-lasting. Hamilton offers four polymeric packing materials for reversed-phase separations.

Type	Recommended Application(s)
PRP-C18	Organic compounds: small molecules (< 2,000 mw), pharmaceuticals, steroids, organic halides, vitamins, amino acid analysis, herbicides
PRP-1	Organic compounds: small molecules (< 2,000 mw), pharmaceuticals, steroids, nucleic acids, vitamins, herbicides
PRP-3	Organic compounds: large molecules (> 2,000 mw), peptides, proteins, protein digests, protected and de-protected oligonucleotides, nucleic acids
PRP-h5	Organic compounds: macromolecules (> 2,000 mw), pharmaceuticals, protein digests, tryptic digests

PS-DVB resins are similar in retention characteristics to silica C18s in that retention tends to increase with lipophilicity. However, subtle differences in the chemical interaction between the analyte and stationary phase can result in differential selectivity. In many cases, analytes that co-elute on a silica C18 can be resolved on a polymeric-based support. The PRP-1 consists of a 55% cross-linked PS-DVB bead containing 100 Å pores. The properties of this base material intrinsically lend itself to reversed-phase separations with no further surface modifications. The PRP-C18 uses the PRP-1 as the base support material with the addition of octadecyl to impart characteristics more closely related to a silica-based C18, giving slightly different selectivity than the PRP-1. To make the PRP-3, the PRP-1 is modified so that the base material contains 300 Å pores, which allows for the separation of larger molecules. The PRP-h5 utilizes the PRP-3 as its base with a pentafluorinated modification, making it more hydrophobic in nature.

In the early stages of reversed-phase chromatography, prototype columns were typically a silica bead functionalized with a C18 chain. These columns were revolutionary for their time but are being replaced by modern polymeric supports.

- PS-DVB supports are as retentive as silica C8 and C18 but offer alternate selectivity
- Stable over the full pH range (1–13)
- Compatible with virtually any aqueous, organic mobile phase
- Can be operated at temperatures well over 85°C
- Improved sample recovery compared to silica-based supports

REVERSED-PHASE HPLC COLUMNS

PRP-C18 Columns

High efficiency separations at any pH

Pore size: 100 Å
Material: C18-functionalized PS-DVB

Mobile phase pH is a powerful tool in methods development, particularly for separation of neutral forms of amines or other organic bases under alkaline conditions. Although some recent C18 columns boast stability in alkaline pH, all silica-based supports experience measurable degradation at pH > 6, where column life is still considerably shorter than if used under more favorable conditions.

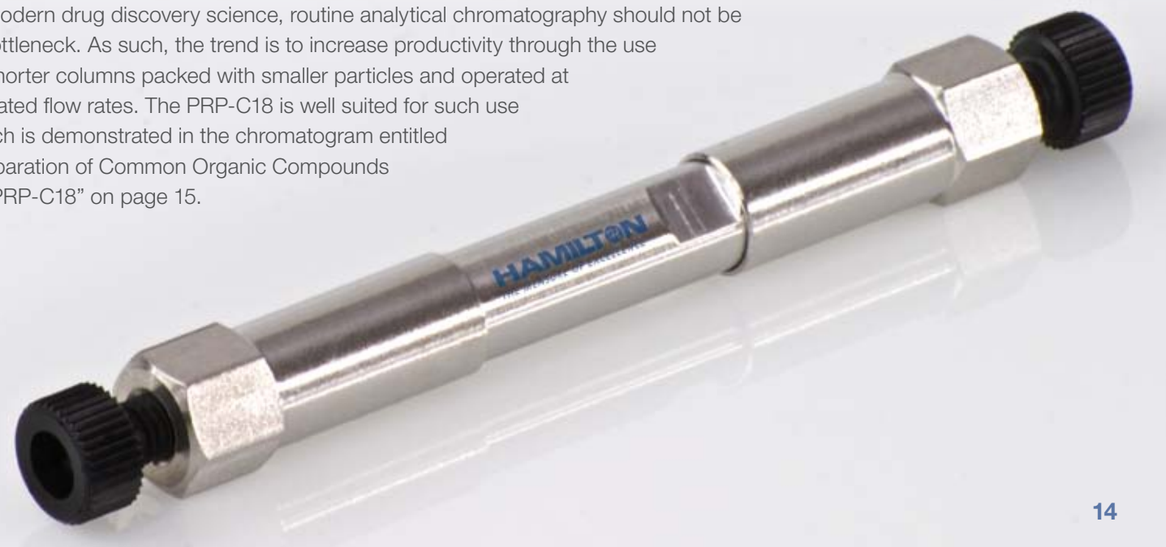
The PRP-C18, on the other hand, has genuine pH and chemical stability. The stationary phase stands up to prolonged exposure to concentrations as high as 1 M NaOH and H_2SO_4, with no measurable decrease in performance. Because the support does not strip, bleed, or dissolve at any pH, it therefore can be expected to perform reliably and reproducibly throughout the extended life of the column, regardless of mobile phase conditions.

High pH applications

More than 70% of all pharmaceutical drug compounds are cationic solutes that carry a formal positive charge below pH 7. Separation of these and other organic bases has historically been problematic. Ionization has a dominating effect in reversed-phase chromatography that tends to dictate retention. Consequently, the elution window for a sample of ionized amines is narrow. The task is further complicated by secondary interactions that occur between positively charged solutes and residual silanols on the column stationary phase. These secondary mechanisms of retention are the principle source for anomalous chromatographic activity, such as poor peak shape, shifts in retention times and loss of efficiency that progressively worsen over the life of the column, as shown in the “Rapid Separation of Basic Drug Compounds on PRP-C18” chromatogram on page 15.

Rapid elution

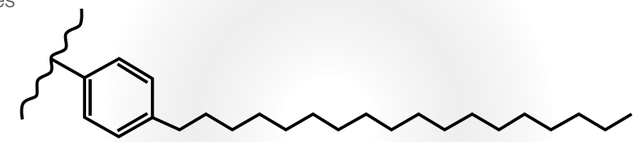
In modern drug discovery science, routine analytical chromatography should not be a bottleneck. As such, the trend is to increase productivity through the use of shorter columns packed with smaller particles and operated at elevated flow rates. The PRP-C18 is well suited for such use which is demonstrated in the chromatogram entitled “Separation of Common Organic Compounds on PRP-C18” on page 15.



PRP-C18 stationary phase structure and applications

Applications:

Organic compounds: small molecules (< 2,000 mw), pharmaceuticals, steroids, halides, vitamins, amino acid analysis, herbicides

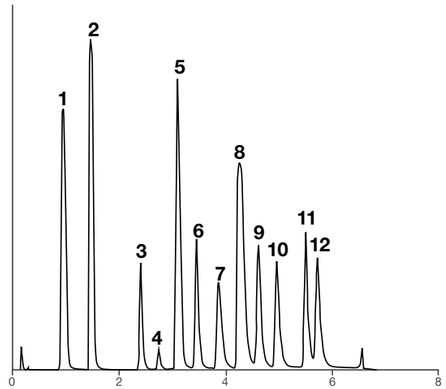


Examples of analytes that can be separated on PRP-C18 columns:

- Peptides
- DNA, RNA oligonucliotides, nucleotides
- Vitamins
- Steroids
- Herbicides
- Pharmaceutical compounds

PRP-C18 application chromatograms

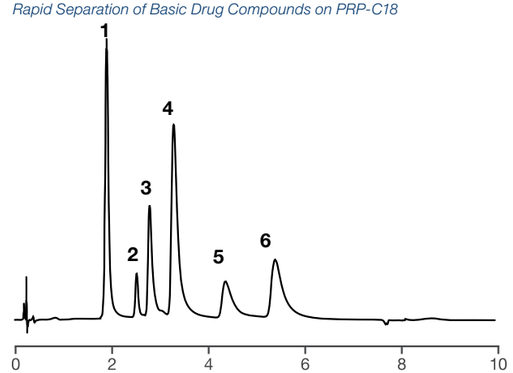
Separation of Common Organic Compounds on PRP-C18



Column: PRP-C18, 4.1 x 50 mm, 5 µm
Part number: 79675
Mobile phase A: Water + 0.2% H_3PO_4
Mobile phase B: Acetonitrile + 0.2% H_3PO_4
Flow rate: 2.5 mL/min
Gradient: 2 to 99% B in 5 minutes
Temperature: Ambient
Injection Volume: 2 µL
Detection: UV at 255 nm

Compounds:
1. Benzamide
2. Nitromethane
3. Nethyl 4-hydroxybenzoate
4. N-ethyl 4-hydroxybenzoate
5. N-propyl 4-hydroxybenzoate
6. N-butyl 4-hydroxybenzoate
7. Benzene
8. Toluene
9. Ethylbenzene
10. Propylbenzene
11. Pentylbenzene
12. Hexylbenzene

Rapid Separation of Basic Drug Compounds on PRP-C18



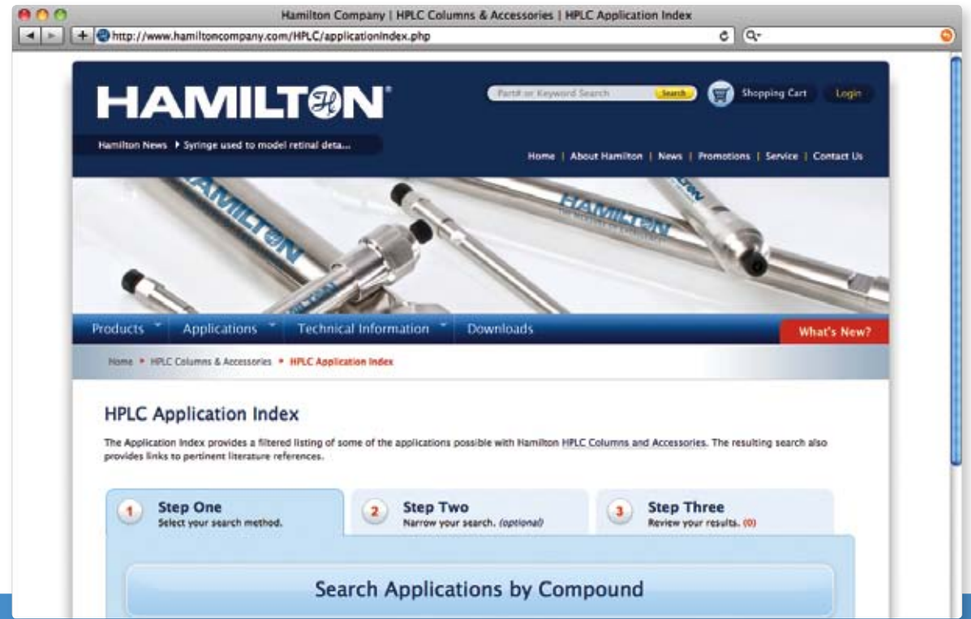
Column: PRP-C18, 4.1 x 50 mm, 5 µm
Instrumentation: Agilent 1100 quaternary pump with UV detector
Standards:
1. Nicotine
2. Metoprolol
3. Quinine
4. Doxylamin
5. Dexmethorphan
6. Amitriptyline

Mobile phase A: 30 mM Diethylamine
Mobile phase B: Mobile phase A + 95% ACN, 5% H2O
Gradient: 10 to 100% B in 5 minutes
Flow rate: 2 mL/min
Temperature: Ambient
Injection volume: 10 µL
Detection: UV at 265 nm

PRP-C18 Column Ordering Information

Dimensions	Particle Size		
	5 µm	10 µm	12–20 µm
2.1 x 50 mm	79672		
2.1 x 50 mm PEEK	79679		
2.1 x 150 mm	79673		
2.1 x 150 mm PEEK	79680		
2.1 x 250 mm	79674		
2.1 x 250 mm PEEK	79681		
4.6 x 50 mm	79675		
4.6 x 50 mm PEEK	79682		
4.6 x 150 mm	79676		
4.6 x 150 mm PEEK	79683		
4.6 x 250 mm	79677		
4.6 x 250 mm PEEK	79684		
21.2 x 250 mm			79678
Bulk Resin (1 gram)	79791	79792	79793

Learn more about PRP-C18 columns at www.hamiltoncompany.com/PRPC18.



View a keyword searchable index of applications possible with Hamilton HPLC columns at www.hamiltoncompany.com/hplcapplicationindex.

PRP-1 Columns

Superior sample recovery

Pore Size: 100 Å
Material: PS-DVB

Sample recovery is vital to sample purification. Problems arise when labile samples become irreversibly bound to the silanol groups present on C8 and C18 HPLC columns. Since Hamilton polymers are made entirely of poly styrene-divinylbenzene, there are no silanol groups to cause sample loss. Recovery and quantitation of labile and reactive samples is enhanced. The purification of protected oligonucleotides demonstrates the enhanced recovery of polymer supports. While approximately 50–80% of an oligonucleotide is recovered on a C18 column, the equivalent PRP-1 column recovers 95% or greater of the same sample.

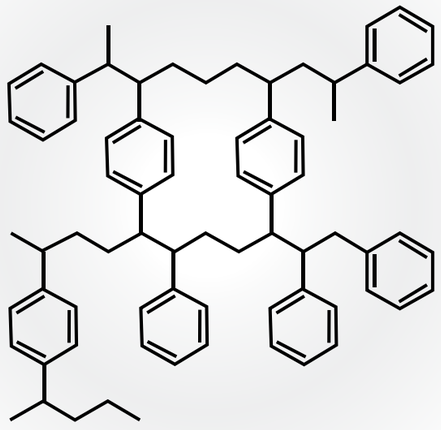
Unlike silica-based C8 or C18 columns, PRP-1 has no stationary phase coating. The integral reversed-phase characteristics of the PRP-1 column eliminate the need for special coating techniques. Since there is no stationary phase to hydrolyze, the column maintains its performance characteristics longer than many C8 or C18 columns.

PRP-1 stationary phase structure and applications

Applications:
Organic compounds: small molecule (< 2,000 mw), pharmaceuticals, steroids, nucleic acids, vitamins, herbicides

Examples of analytes that can be separated on PRP-1 columns:

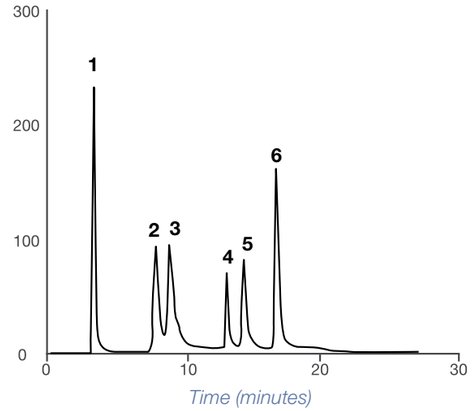
- Polycyclic aromatic hydrocarbons (PAH)
- Ionizable organic compounds
- Steroids
- Peptide fragments



Guard columns are an easy way to prolong an analytical column's life. Refer to page 49 for more information on how guard columns protect your investment.

PRP-1 application chromatograms

Separation of Adenine and Guanine Nucleotides on PRP-1



Column: PRP-1, 5 µm, 2.1 x 150 mm
Part number: 79366
Mobile phase A: 100 mM Monopotassium phosphate (adjust pH to 7 with potassium hydroxide), 1 mM tetrabutylammmonium phosphate, 2.5% methanol
Mobile phase B: Mobile Phase A + 20% methanol
Flow rate: 300 µL/min
Gradient:

Time (min)	%B
0–3	1
10	15
15	55
16–19	95

Injection volume: 5 µL
Sample concentration: 0.02 mM
Temperature: 50°C
Detection: UV at 254 nm

Compounds:
1. Guanosine monophosphate
2. Guanosine diphosphate
3. Adenosine monophosphate
4. Guanosine triphosphate
5. Adenosine diphosphate
6. Adenosine triphosphate

PRP-1 Column Ordering Information

Hardware Dimensions	Particle Size					
	5 µm	7 µm	10 µm	12–20 µm	30–50 µm	50–75 µm
1.0 x 50 mm		79755				
1.0 x 150 mm	79753					
2.1 x 100 mm	79790					
2.1 x 150 mm	79366					
4.1 x 50 mm	79443					
4.1 x 100 mm	79479					
4.1 x 150 mm	79444	79529	79425			
4.1 x 250 mm	79820	79422	79427			
4.6 x 100 mm PEEK	79558					
4.6 x 150 mm PEEK	79423		79351			
4.6 x 250 mm PEEK	79571	79380	79381			
7.0 x 100 mm			79495			
7.0 x 305 mm	79795		79426			
10.0 x 50 mm		79367				
10 x 100 mm	79355		79499			
10 x 250 mm		79531	79496			
21.2 x 75 mm	79154					
21.2 x 250 mm		79352	79478	79428		
30 x 250 mm				79229		
50 x 250 mm			79567	79493		
101.6 x 250 mm				79525		
101.6 x 250 mm Repack				79800		
Bulk Resin (1 Gram)	79578	79579	79580	79581	79582	79583

PRP-3 Columns

Reversed-phase column optimized for separation of macromolecules such as DNA, RNA oligos, proteins, peptides and proteomics

Pore size: 300 Å
Material: PS-DVB

Hamilton's PRP-3 is a polymeric reversed-phase HPLC column designed for the purification and isolation of proteins and peptides with very good recovery (> 90%). It is based off of the PRP-1 but utilizes a 300 Å pore size rather than the PRP-1's 100 Å pore size. The highly inert polymeric packing poly(styrene-divinylbenzene) enhances protein recovery because there are no silanol groups on the support to cause irreversible protein adsorption.

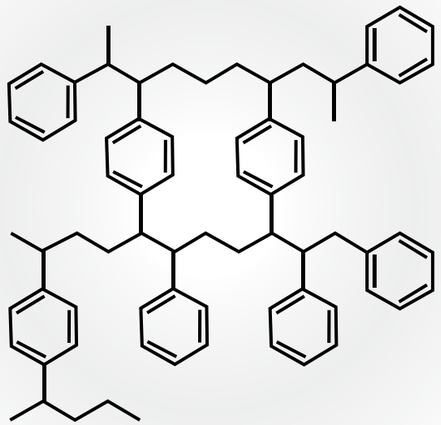
PRP-3 is a PS-DVB support that is pressure stable up to 5,000 psi and cross-linked to prevent shrinking or swelling when the mobile phase is changed. Chemically, proteins present solubility problems unlike many small molecules. Most proteins are hydrophobic on the inside, with highly charged exteriors. This often presents dissolution problems, particularly when pH is near the isoelectric point of the protein. The rugged chemical nature of the PRP-3 allows the protein chemist a much broader selection of agents for dissolution, including concentrated acids, aggressive chaotropes, as well as detergents. In proteomics, the PRP-3 has excellent potential for single-column 2D HPLC. The orthogonal selectivity between low and high pH separations is often comparable to that achieved with two different column formats (SCX, RP), but with the added bonus of MS-compatible mobile phase.

PRP-3 stationary phase structure and applications

Applications:
Organic compounds: large molecules (> 2,000 mw), peptides, proteins, protein digests, protected and de-protected oligonucleotides, nucleic acids

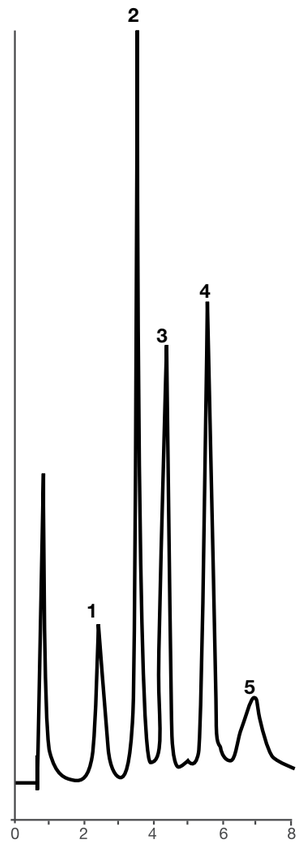
Examples of analytes that can be separated on PRP-3 columns:

- Globular proteins
- Albumins
- Antibody fragments
- Tryptic digests
- DNA
- RNA oligomers
- Synthetic high mw polymers



PRP-3 application chromatograms

Five Proteins on PRP-3



Time (minutes)

Column: PRP-3, 4.1 x 150 mm, 10 µm
Part number: 79466
Standards:
1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. Myoglobin
5. Ovalbumin
Mobile phase A: 0.01% TFA in water pH 2.0
Mobile phase B: 0.1% TFA in acetonitrile
Gradient: 25 to 50% B in 5 min. Hold 3 min.
Flow rate: 2 mL/min
Temperature: Ambient
Injection volume: 100 µL
Detection: UV at 215 nm

PRP-3 Column Ordering Information

Hardware Dimensions	Particle Size	
	10 µm	12–20 µm
2.1 x 150 mm	79392	
4.1 x 150 mm	79466	
4.6 x 150 mm PEEK	79382	
4.6 x 250 mm PEEK	79574	
7.0 x 305 mm	79468	
10 x 250 mm	79526	
21.2 x 250 mm	79147	
21.2 x 100 mm		79186
21.2 x 250 mm		79469
Bulk Resin (1 gram)	79701	79702

A selection of column hardware sizes is available from analytical to semi-prep and preparative. Sample scale up is easy because the PRP-3 packing is consistent from analytical to preparative columns. This saves time and eliminates the need to redevelop separations on semi-prep or preparative columns. The short analytical (50 mm) column is well-suited for the gradient elution of high molecular weight proteins and the longer (150 mm) column is best for smaller proteins.

Custom HPLC columns are available! From dimensions to particle size to packing materials, Hamilton can build you exactly what you need. *See page 48 for more information.*

PRP-h5 Columns

Pentafluoro reversed-phase column for unique selectivity

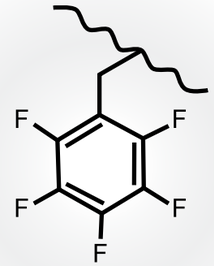
Pore size: 300 Å
Material: Pentafluorinated PS-DVB

The PRP-h5 utilizes the PRP-3 as its base with a pentafluorinated modification, making it more hydrophobic in nature. The PRP-h5, with its functionality derived from a pentafluorinated polymer bead, delivers a selectivity difference from standard silica C18 stationary phases. This gives chromatographers the desired retention characteristics necessary for certain sample types. This difference is especially pronounced for halogenated solutes.

Unlike silica-based C8 or C18 columns, PRP-h5 has no stationary phase coating. Since there is no stationary phase to hydrolyze, the column maintains its performance characteristics longer than many silica-based C8 or C18 columns.

PRP-h5 stationary phase structure and applications

Applications:
Organic compounds: macromolecules (> 2,000 mw), pharmaceuticals, protein digests, tryptic digests, proteomics

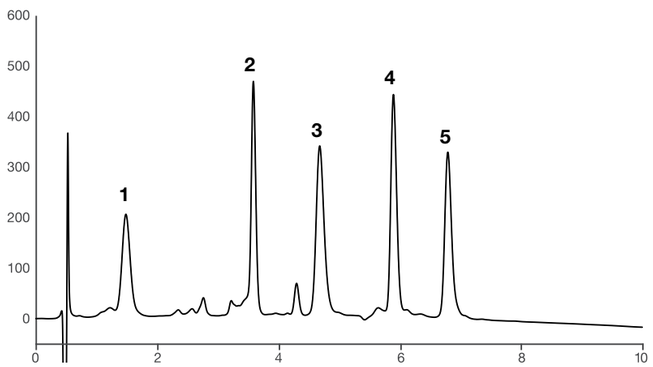


Examples of analytes that can be separated on PRP-h5 columns:

- Oligonucleotides
- Angiotensin
- apo-Transferrin
- Apomyoglobin (equine)
- Carbonic anhydrase
- Cytochrome C
- Myoglobin
- Ribonuclease A

PRP-h5 application chromatograms

Five Proteins at 80°C on PRP-h5



Column: PRP-h5, 4.6 x 50 mm, 5 µm
Part number: 79261
Standards:
1. Ribonuclease A
2. Cytochrome C
3. apo-Transferrin
4. Myoglobin
5. Carbonic Anhydrase
Mobile phase A: DI Water, 0.1% TFA
Mobile phase B: Acetonitrile, 0.1% TFA
Gradient: 22 to 55% B in 10 min.
Flow rate: 1 mL/min
Temperature: 80°C
Injection volume: 15 µL
Detection: UV at 210 nm

REVERSED-PHASE HPLC COLUMNS

PRP-h5 Column Ordering Information

Hardware Dimensions	Particle Size 5 µm	12–20 µm
2.1 x 100 mm	79270	
2.1 x 150 mm	79271	
4.6 x 50 mm	79261	
4.6 x 100 mm	79262	
4.6 x 150 mm	79272	
4.6 x 250 mm	79273	
10 x 100 mm	79263	
10 x 150 mm	79274	
Bulk Resin (1 gram)	79269	79280

Hamilton is your partner. From determining the correct column for your application to post-purchase support and troubleshooting, the HPLC team is standing by and ready to help.